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Original Article

Prevalence of Hepatitis C virus among Out-Patients of a Private Laboratory in Tehran

*M Hajia[1, 4], N Shahrokhi[2, 4], AA Amirzargar [3, 4], M Farzanehkhah[4], S Biglari[4], M Ghorishy[4], *A Sarafnejad [5, 4]*

[1]*Research Center of Reference Laboratories of Iran, Tehran, Iran*
[2]*Dept. of Molecular Biology, Pasteur institute of Iran, Tehran, Iran*
[3]*Dept. of Immunology, Medical School, Tehran University of Medical Sciences, Iran*
[4] *Noor Pathobiology Laboratory, Tehran, Iran*
[5]*Dept. of Immunology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

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Abstract

With an estimated 170 million infected individuals, hepatitis C virus (HCV) has a major impact on public health. Frequency of HCV infection was studied in 785 referred patients to a private Laboratory, Tehran, Iran from April 2003 until March 2006 by PCR method. Analyzed results revealed infection rate was 40.27%, 35.51% and 33.09% in the first, second and third year, respectively. Frequency rate of the infection shows a fluctuated shape during months of three years. Maximum rate of the HCV infection was in May of the first and in June of the second and third year, respectively. It shows decrease and increase within rest months in each year. HCV infection rate was higher in 31 to 40 years old group of patients. Analysis of the data revealed higher HCV infection in men than women had. It was concluded that HCV frequency pattern underlines specific attention to suspected patients during high rate time.

Keywords: *Prevalence, HCV infection, Diagnosis, Iran*

Introduction

HCV is one of the important causative agents of hepatitis and is the most cause for liver graft in many countries (1). It has been postulated that the frequency of this infection is 3-5% with over 170 million of the world population (2). Hepatitis C virus is transmitted via blood, and can survive up to three days in dried blood and cause infection by any accidentally contact (3).

HCVcAg is informative qualitative index for HCV viremia. Serology and Western Blot assay are two other applied methods that each has some limitations. Specific HCV antibody will not rise in early acute phase of infection but will remain positive in the clearance phase. False positive results are also reported frequently. Another test that is used in the clinical laboratory as a screening test is western blot assay. This test detects specific antibodies based on structural and non-structural antigens. It has high specificity that is negative in the acute phase and is positive in the clearance period as well (4,5). This test is used as confirmatory assay for the presence of specific HCV antibodies. HCV RNA is found in 69.5% among 177 HCV positive samples by RIBA (6).

It is recommended that all patients with suspected chronic HCV infection should be tested for HCV RNA, since seropositivity without detectable viremia is frequent (7). Today, PCR is the most trustable method in diagnosis, indicating viral load and genotyping of HCV infection. This method will be positive in early acute phase of infection (8). It is possible to evaluate the treatment progress by estimation of the viral load. Clinicians are able to choose efficient treatment period by PCR genotyping reports as well (9). The aim of this study was to determine HCV positive rate among suspected patients by PCR within three years of study period.

***Corresponding author:** Fax: +98 21 66422337, E-mail: saraf@sphtums.com

Materials and Methods

Patients and samples All those suspected patients who had signs and symptoms of hepatitis, were referred to the Noor Pathobiology laboratory for HCV PCR. 785 sera specimens were collected either from out-patients or from 300 laboratories allover the country during Apr 2003 until Mar 2006. Yearly received samples were 216, 288 and 281 in the first, second and third year of the study, respectively. These samples were kept at -20 ^{o}C immediately for a couple of days in maximum and defreezed before commencing extraction.

RNA extraction Clinical samples were extracted by High Pure Viral Nucleic Acid Purification kit (Viral extraction kit of Roche).

RT-PCR protocols cDNA was immediately prepared with provided protocol of HCV PCR kit (DNA Technology Co). After extraction of RNA use of MTC410 thermocycler (DNA Technology Co) at 37 ^{o}C for 45 min followed by at 75 ^{o}C for 5 min.

One hundred U reverse transcriptase, 16.5 µl extracted RNA and 2-µl RT buffer were added to the provided mixture by kit, containing random hexamer and dNTP to make the cDNA. Specific primers target core protein gene of HCV genome and amplified 153 base pair of the made cDNA. Five µl of prepared cDNA, 10 µl PCR buffer, 10 µl mixture containing specific primers and dNTP, 2.5 U taq polymerase was then mixed and amplified with the specific programme (Table 1). Provided specific primers can also amplify a 900 base pair of internal control to ensure of proper extraction and removal of any expected inhibitors. The kit contains specific labeled probe enable us to detect the amplified by the fluorescence detector (DNA Technology) called Fluorescent Amplification-based Specific Hybridization method (DNA Technology). Preparation steps of RT PCR were performed on ice; extracted RNA and prepared cDNA were immediately used in the test.

Results

Extraction results Different extraction protocols had various recovery rates for HCV RNA. In this study three extraction kits were compared.. Applied extraction methods were High Pure Viral Nucleic Acid Purification (HPVNP), High Pure Template Preparation (HPTP) and RNX plus kits (Roche). The main difference between these two is use of polyA as a carrier. The third extraction method was the use of RNX Plus (Cinnagen Co). Both HPVNP and RNX plus gave higher sensitivity. Our experiments proved ignoring proteinase K in extraction process do not affected on the HCV RNA recovery rate.

Study on the HCV positive rate revealed its frequency pattern was nearly similar in three studied years. It increased in the late spring and early of the summer, and decreased for a while. HCV rate again started to rise from autumn and decreased gradually in the winter (Table 2 and Fig. 1). Similarity of the HCV distribution within a year of studied period also confirms the validity of the results for later analysis of HCV infection.

Analysis of the results also indicated positive rate was decreased slightly in these three years (Table 3). HCV infection rate was higher in men than women. Number of suspected women was nearly about one third of the total referred patients, while women with proved HCV infection had obviously lower rate than men about one forth (Table 4).

Table 1: Applied Amplification program for HCV RT-PCR

Temperature (°C)	Time (seconds)	Repeat
94	180	1
94	50	5
64	50	
72	30	
95	30	40
64	50	
67	20	
10	Storage	

Table 2: Frequency of HCV positive rate from April 2003 till March 2006

Month	Apr 2003-Mar 2004	Apr 2004-Mar 2005	Apr 2005-Mar 2006
April	14.88	46.15	13.33
May	66.66	38.46	30
June	47.05	62.5	43.4
July	47.61	25.92	22.7
August	50	16	34.7
September	26.66	26.82	30.4
October	45.45	53.57	52.3
November	33.33	46.15	30
December	50	47.36	45.4
January	32.14	34.61	43.7
February	26.08	31.81	30.4
March	27.27	15.76	24.2

Table 3: HCV positive rate in April 2003 till March 2004

	April 2003- March 2004	April 2004- March 2005	April 2005- March2006
Positive rate	89 (40.27%)	103 (35.51%)	93 (33.09%)
Negative rate	132 (59.73%)	187 (64.49%)	188 (66.91%)
Total cases	221	290	281

Table 4: Frequency of HCV infection in men and women

	April 2003- March2004		April 2004- March2005		April 2005- March2006	
	n(%) of received samples	n(%) of positive samples	n(%) of received samples	n (%) of positive samples	n (%) of received samples	n(%) of positive samples
Women	54 (24.43)	13 (14.61)	81(27.93)	22 (21.35)	72 (25.63)	14 (15.05)
Men	167 (75.57)	76 (85.39)	209(72.07)	81 (78.64)	209 (74.37)	79 (84.95)
Total	221	89	290	103	281	93

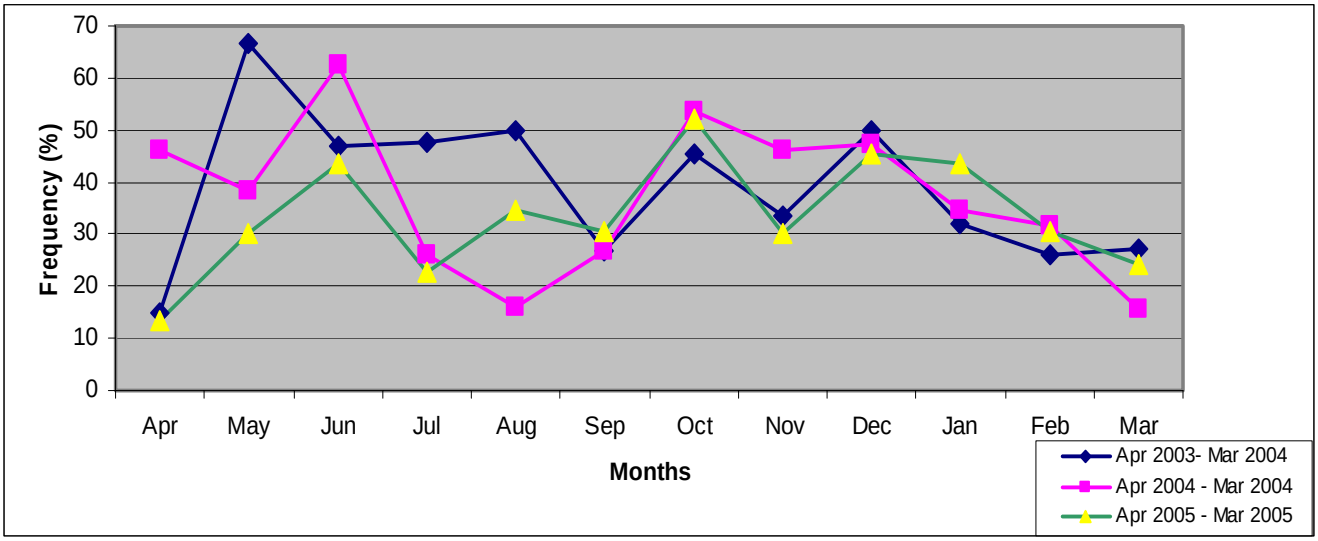

Fig. 1: Frequency of HCV positive rate from April 2003 till March 2006

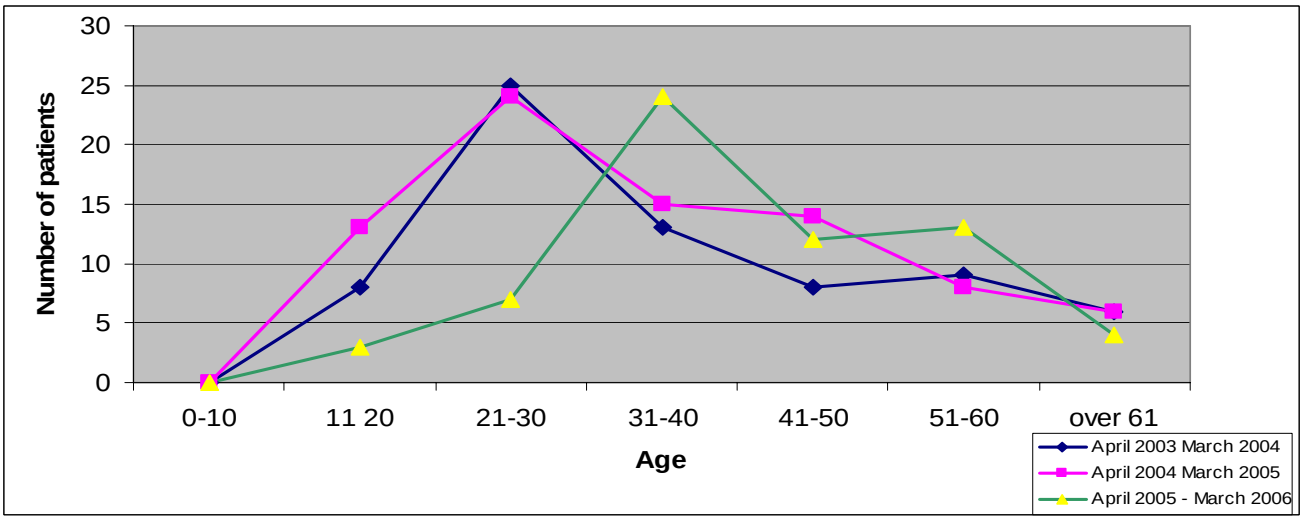

Fig. 2: Frequency of HCV positive rate in different age

Discussion

Unfortunately, there have been no reports on the prevalence of HCV infection in Iranian population. There are some specific reports on drug abuse (10), hemodialysis patients (11, 12), and street children. These reports are not good preventative for HCV infection estimation in the general population (13). It was quite important for us to know the prevalence of the hepatitis C especially during the year, and between men and women. These patients were a suitable suspected population of the community because of their sign and symptom since there was no any selection on them. In spite of this point, the pattern of HCV frequency was obviously similar during all three years of study period (Fig.1)

The highest HCV rate was observed in patients with 21-30 yr old in both Apr 2003 until Mar 2004 & Apr 2004 until Mar 2005. However, its frequency changed among different age groups

in April 2005 until March 2006. Patients with 31-40 yr old had the highest frequency rate. Chandra has reported similar rate in India as well (14).

Different reports confirm HCV infection rate is observed mostly in adult people with 20-40 yr old. Our data also shows similar pattern, although it seems its frequency rate has changed slightly in 2001. HCV infection is obviously expected to be rare in infant according to Fig. 2.

Risk factors for HCV infection include intravenous drug use, transfusion of blood products, hemodialysis, tattooing, high-risk sexual behavior, exposure to health care, and organ transplants from HCV-positive donors. Alavian et al. (15) had a case-control study on the blood donor to the Iranian Blood Transfusion Organization and underlined the effect of some factors such as transfusion, undergoing endoscopy, extramarital sexual activities, non-intravenous drug abuse on the prevalence of the hepatitis C infection there were no risk factors. They reported 24.5% of the positive cases had no apparent risk factors. Specific HCV antibody is less than 2% of the general population in some countries (16). Unfortunately, there is no report about status of the HCV in Iran. However, its rate is obviously higher in men. It seems to be necessary to investigate on the probable HCV risk factors to reduce the infection rate in this group. It is concluded that PCR technique is a powerful method for diagnosis HCV infection. Analysis of the results HCV infection is higher in adults and its frequency in men is about four times more than women.

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